Y-chromosomal background of gr/gr deletions

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INTRODUCTION

The gr/gr deletion is a common Y chromosome abnormality that removes 1.6Mb of the AZFc region including two DAZ and one CDY1 gene copies (Figure 1). The relationship between this deletion and male infertility is a subject of a continuing intense debate. The gr/gr deletion is regarded as a risk factor for reduced sperm counts in some populations, but not in others.

MATERIALS AND METHODS

We screened a total of 740 men from the Republic of Macedonia (520 Macedonians, 140 Albanians and 80 Roma, Serbs, Turks and Croats) for the presence of gr/gr deletion. The methodology for detection and characterization of gr/gr deletion included analysis of DAZ, MYPT2Y, CDY1 and CDY2 gene copy number by quantitative fluorescent (QF)-PCR analysis; PCR analysis of several sequence tagged site (STS) markers; and analysis of DAZ and CDY1 specific single nucleotide variants (SNV) by PCR and restriction fragment length polymorphism (RFLP).

The Y chromosome haplogroups were determined by 32 Y-chromosome SNP markers, which were typed following a hierarchical method, in five multiplex PCR/SNaPshot reactions (Figure 2).

RESULTS

The detection of gr/gr deletion by STS analysis is shown in Figure 3; it is characterized by absence of sY1291 and presence of sY1191. QF-PCR analysis allowed for detection of gr/gr deletions with b2/b4 duplication, a rearrangement that has probably arisen from gr/gr deletion followed by duplication (Figure 4). The gr/gr deletion is characterized by reduced DAZ/DAZL and MYPT2Y/MYPT2 ratios and increased CDY1/CDY2 ratios. The gr/gr deletion with b2/b4 duplication is characterized by normal or increased DAZ/DAZL and CDY1/CDY2 ratios, and reduced to half MYPT2Y/MYPT2 ratio.

We found 21 men with gr/gr deletion and gr/gr deletion-b2/b4 duplication (Table 1). Nineteen of the 21 men with gr/gr deletion were Macedonians, one was Serb and one was Croat. No gr/gr deletion was detected among the 140 Albanians. There was a statistically significant difference (p=0,0217) in the incidence of gr/gr deletion between Macedonian (3,65%) and Albanian males (0%).

Nine different Y chromosome haplogrous were determined among men with gr/gr deletions, three of which (I2a-P37b, R1a1-SRY1532 and J2b2-M241) are among the five most common haplogroups in the Republic of Macedonia. The most frequently deleted DAZ copy was DAZ1/2, whereas the predominantly missing CDY1 copy was CDY1b. There was a difference in the haplogroup distribution of carriers with the loss of CDY1a and CDY1b (Figure 5).

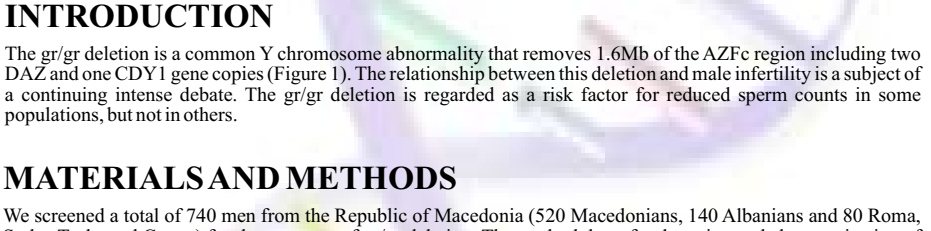

Figure 1. Schematic representation of the gr/gr deletion in the AZF locus on Y chromosome.

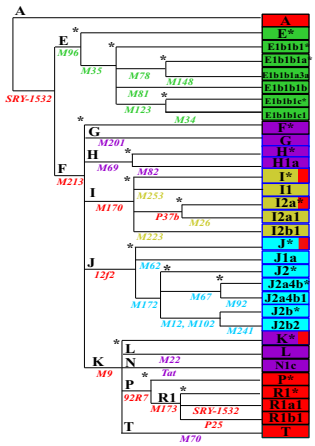

Figure 2. Phylogenetic Y chromosome tree defined with the 32 SNPs analyzed. Marker names are shown above the lines. Colors represent multiplex groups.

Table 1. Findings in the 21 males with gr/gr deletion.

No.	Sample ID	Ethnic origin	Fertility status	STS		DAZ SNVs				CDY1 -SY30	Gene copy number (ratio)			Deletion type	Y-Hgr
				sY 1291	sY 1191	SY 587 T/C	SY 581 C/T	SY 586 C/T	DAZ SNV II G/A	C/A	DAZ/ DAZL	MYPT2/ MYPT2Y	CDY2/ CDY1		
1	MI 7	Macedonian	oligozoospermia >5	absent	present	C	T	C	A	C	0.8	1.8	2.0	gr/gr del	I2a
2	MI 38	Macedonian	azoospermia	absent	present	C	T	C	A	A	1.0	2.8	2.1	gr/gr del	E1b1b1c1
3	MI 41	Macedonian	normozoospermia	absent	present	C	T	C	A	A	2.3*	2.3	1.0	gr/gr del+b2/b4 dup	J2a4b1
4	MI 99	Macedonian	azoospermia	absent	present	T, C	C, T	C	G, A	C	1.5	2.6	1.9	gr/gr del	I2a
5	MI 137	Macedonian	normozoospermia	absent	present	T	C	C, T	G	C	1.1	2.3	2.0	gr/gr del	T
6	MI 146	Macedonian	normozoospermia	absent	present	T	C	C, T	G	C	1.0	2.9	2.1	gr/gr del	T
7	MI 203	Macedonian	normozoospermia	absent	present	C	T	C	A	C	1.1	2.3	1.8	gr/gr del	I2a
8	MI 215	Macedonian	oligozoospermia <5	absent	present	C	T	C	A	C	0.7	2.6	1.8	gr/gr del	R1a1
9	CF17/T	Macedonian	Fertile	absent	present	T, C	C	C	G, A	A	2.2*	2.4	1.9	gr/gr del+b2/b4 dup	K
10	CF158/T	Macedonian	Fertile	absent	present	C	T	C	A	A	2.9*	2.0	0.9	gr/gr del+b2/b4 dup	J2a4b1
11	CY21	Serb	Fertile	absent	present	T	C	C	G	A	1.0	2.8	2.2	gr/gr del	I2b1
12	H41/T	Macedonian	Fertile	absent	present	T, C	C	C	G	A	0.7	2.7	2.0	gr/gr del	R1a1
13	R4137/T	Macedonian	Fertile	absent	present	C	T	C	A	A	0.8	2.2	2.0	gr/gr del	R1a1
14	SM 83/T	Macedonian	Fertile	absent	present	C	T	C	A	C	0.8	2.7	1.9	gr/gr del	I2a
15	CC 44	Macedonian	Unknown fertility	absent	present	C	T	C	A	C	0.9	2.5	2.1	gr/gr del	I2a
16	CC 275	Macedonian	Unknown fertility	absent	present	/	/	/	/	/	0.8	2.0	1.9	gr/gr del	/
17	CC 321	Macedonian	Unknown fertility	absent	present	/	/	/	/	/	1.1	2.2	1.9	gr/gr del	/
18	HLAS 73	Macedonian	Unknown fertility	absent	present	C	T	C	A	C	0.9	2.1	1.8	gr/gr del	R1a1
19	PC 182	Croat	Unknown fertility	absent	present	C	T	C	A	C	1.0	2.4	2.0	gr/gr del	R1a1
20	PC 209	Macedonian	Unknown fertility	absent	present	C	T	C	A	C	0.8	2.0	2.1	gr/gr del	A
21	PC 386	Macedonian	Unknown fertility	absent	present	C	T	C	A	A	0.7	2.3	1.9	gr/gr del	J2b2
						DAZ1/2-T DAZ3/4-C	DAZ1/4-C DAZ2/3-T	DAZ1/3/4-C DAZ2-T	DAZ1-G DAZ2/3/4-A	CDY1a-C CDY1b-A	Normal ratios 2		1		

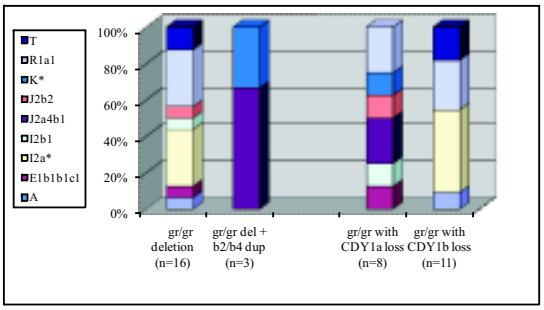

Figure 5. The distribution of Y haplogroups in men with gr/gr deletion.

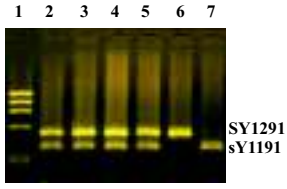

Figure 3. Agarose gel electrophoresis of the duplex PCR of sY 1291 and sY1191 STS markers. 1) DNA ladder; 2-5, normal male DNA samples; 6, DNA from a men with b2/b3 deletion, 7) DNA from a men with gr/gr deletion.

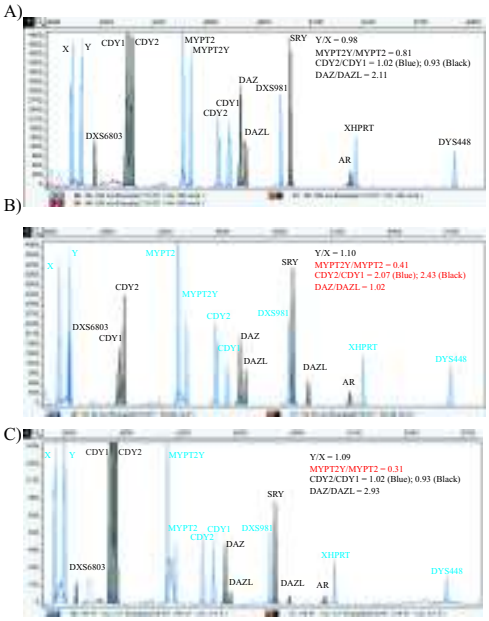

Figure 4. Electrophoreograms of QF-PCR analysis from normal male (A) and males with gr/gr deletion (B) and gr/gr deletion + b2/b4 duplication (C).

CONCLUSIONS

The ethnic difference in the incidence of gr/gr deletion found in our study may have relevance for the interpretation of case control studies dealing with admixed populations.

ACKNOWLEDGMENTS

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